A streamlined LC-MS/MS platform for the screening and confirmation of 152 veterinary drug residues in a broad range of food raw materials, processed ingredients and finished products

Nestlé Research Centre, Lausanne, Switzerland

Thierry DELATOUR, Aurélien DESMARCHELIER, Marie-Claude SAVOY, Adrienne TARRES, Claudia MUJAHID, and Andrea BECK, Thomas BESSAIRE & Pascal MOTTIER

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Veterinary Drugs

Definition

Any substance applied or administrated to any food-producing animal, such as meat or milk producing animals, poultry, fish or bees, whether used for therapeutic, prophylactic, or diagnostic purposes, or for modification of physiological functions or behaviour.

Use

To **treat existing illness**, to **prevent future disease**, and **promote growth**.

Pharmacological actions

ANTI biotics
inflammatories
parasitics

Tranquiliz
Painkill
Growth-promot **ERS**

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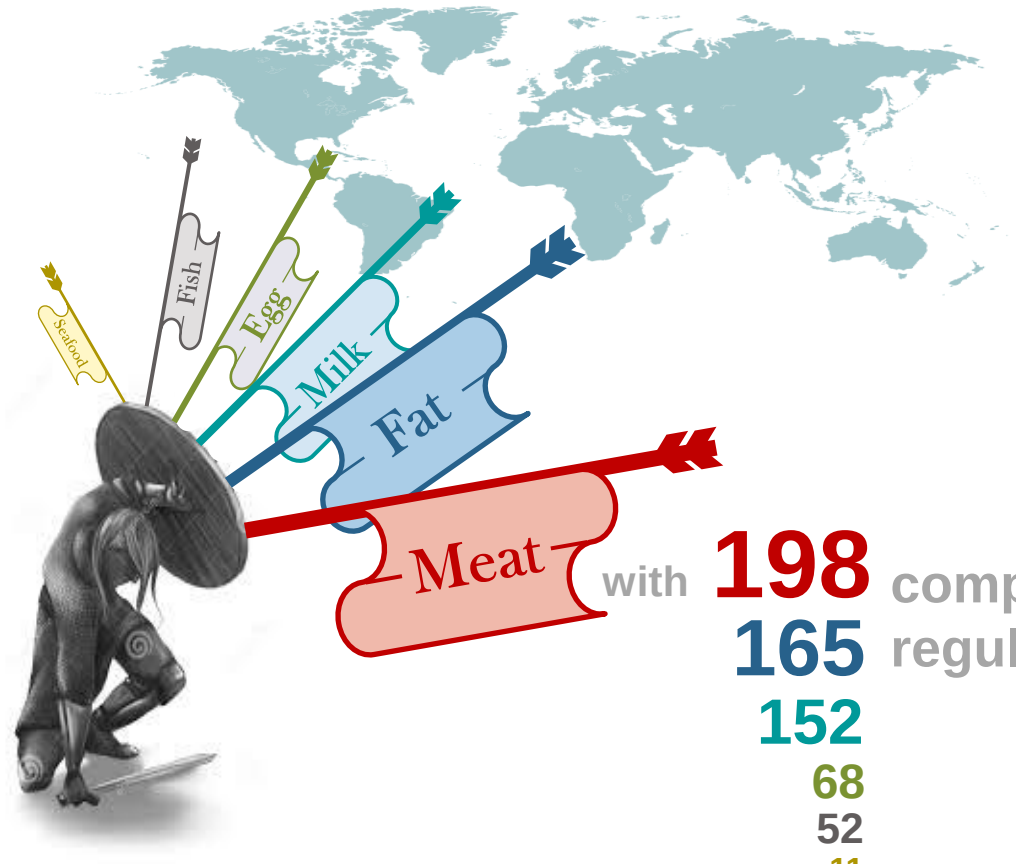
Regulatory framework



with **198** compounds

165 regulated

152

68

52

11

Source: *EU, USA, Canada, China, Codex Alimentarius*

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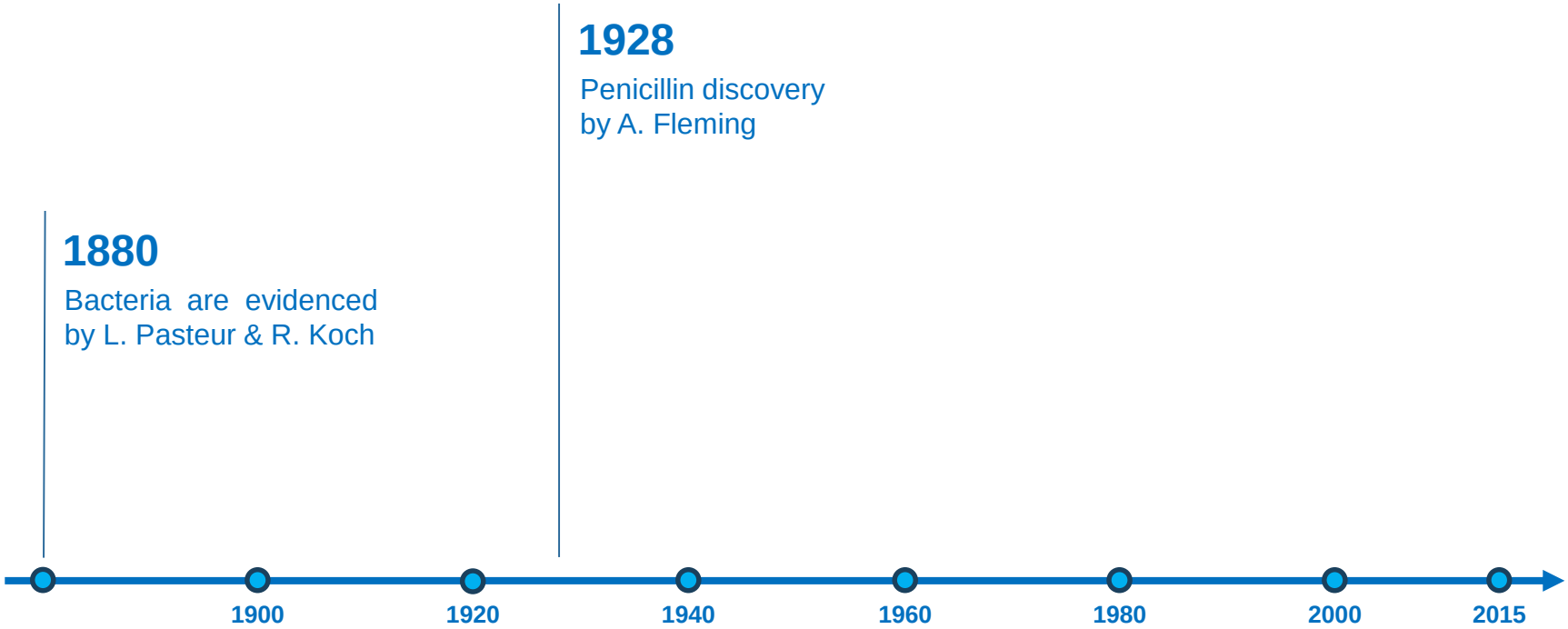
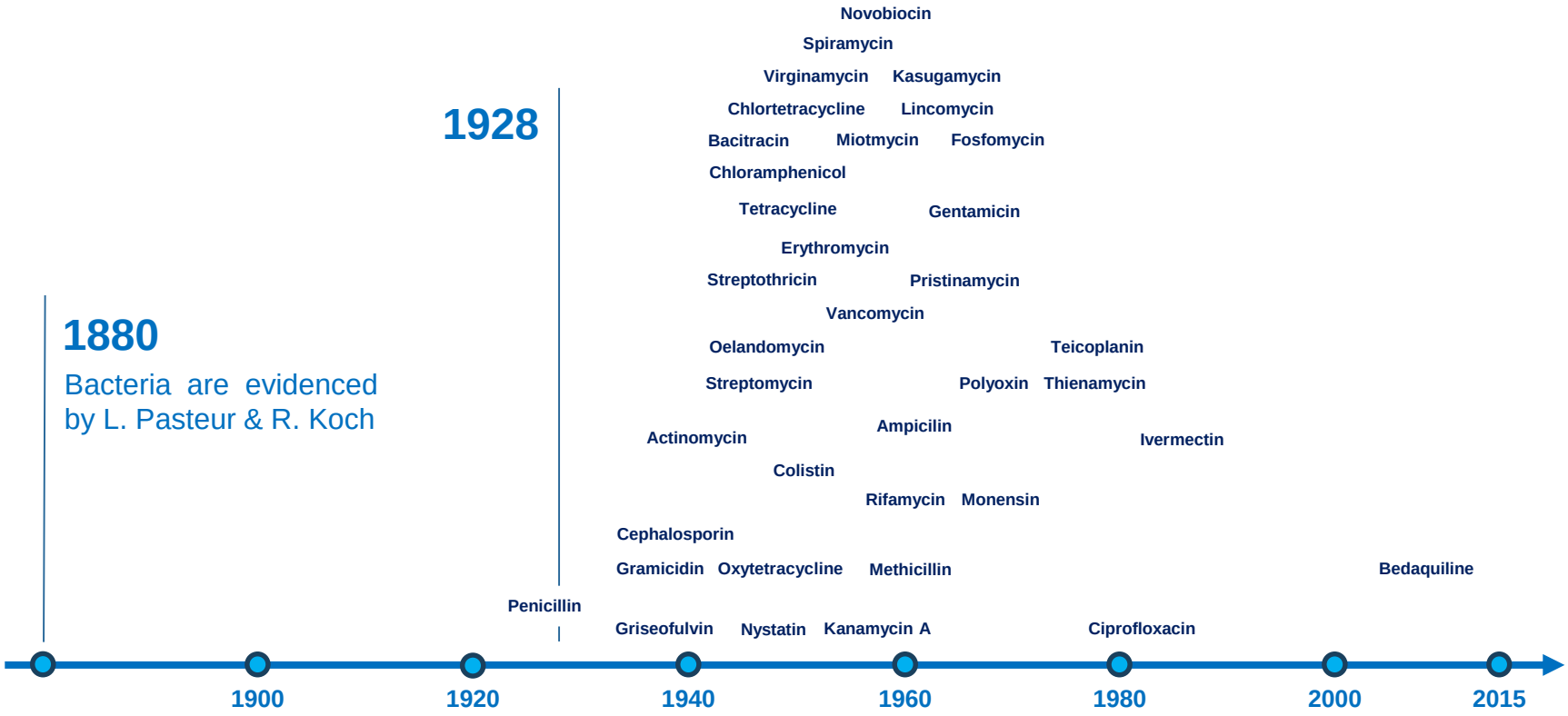
Antibiotics at a glance



Source: Le Monde, 2017, La santé & la médecine.

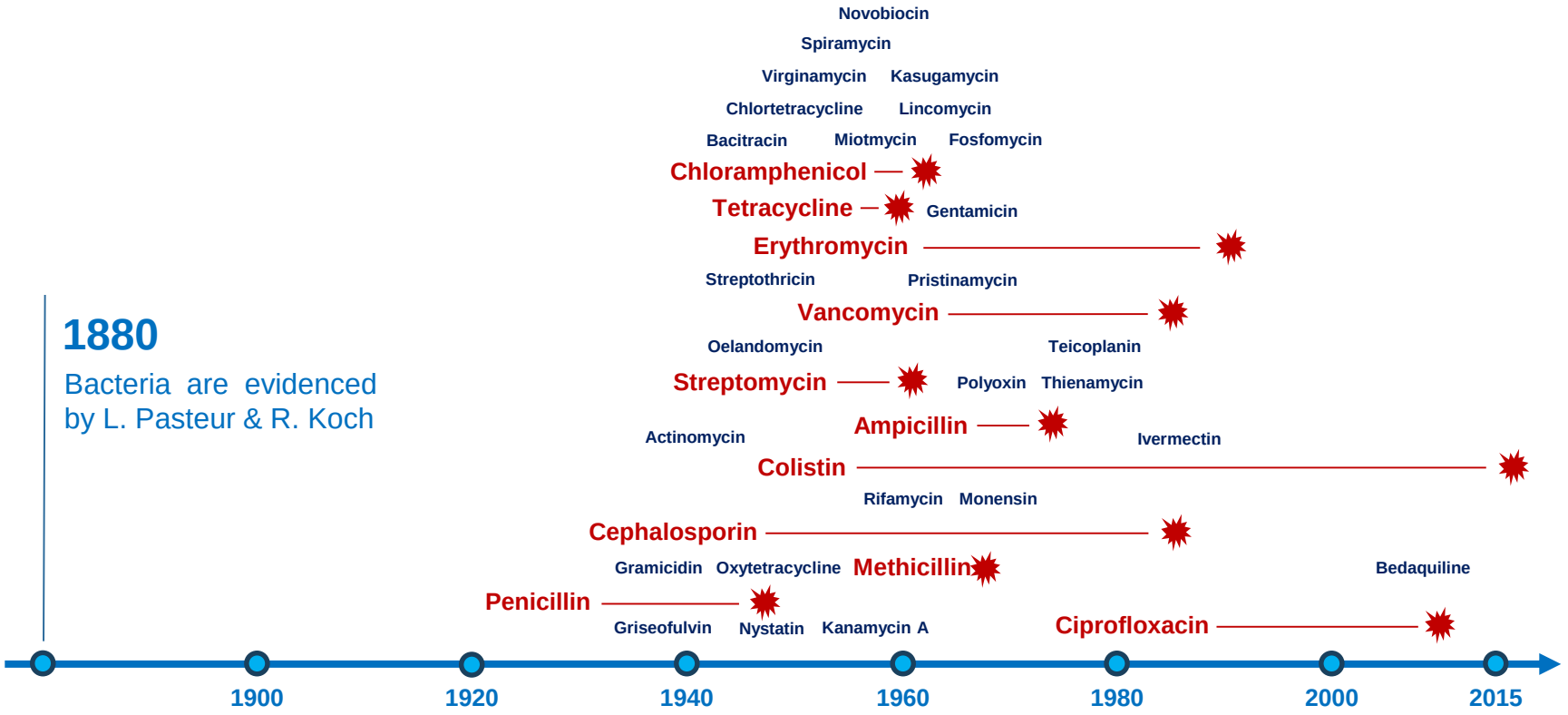
Antibiotics at a glance



Source: Le Monde, 2017, La santé & la médecine.

Antibiotics at a glance

First antimicrobial resistance evidenced



Source: Le Monde, 2017, La santé & la médecine.

Food materials

- **Milk**
- **Meat**
- **Fish/seafood**
- **Egg**

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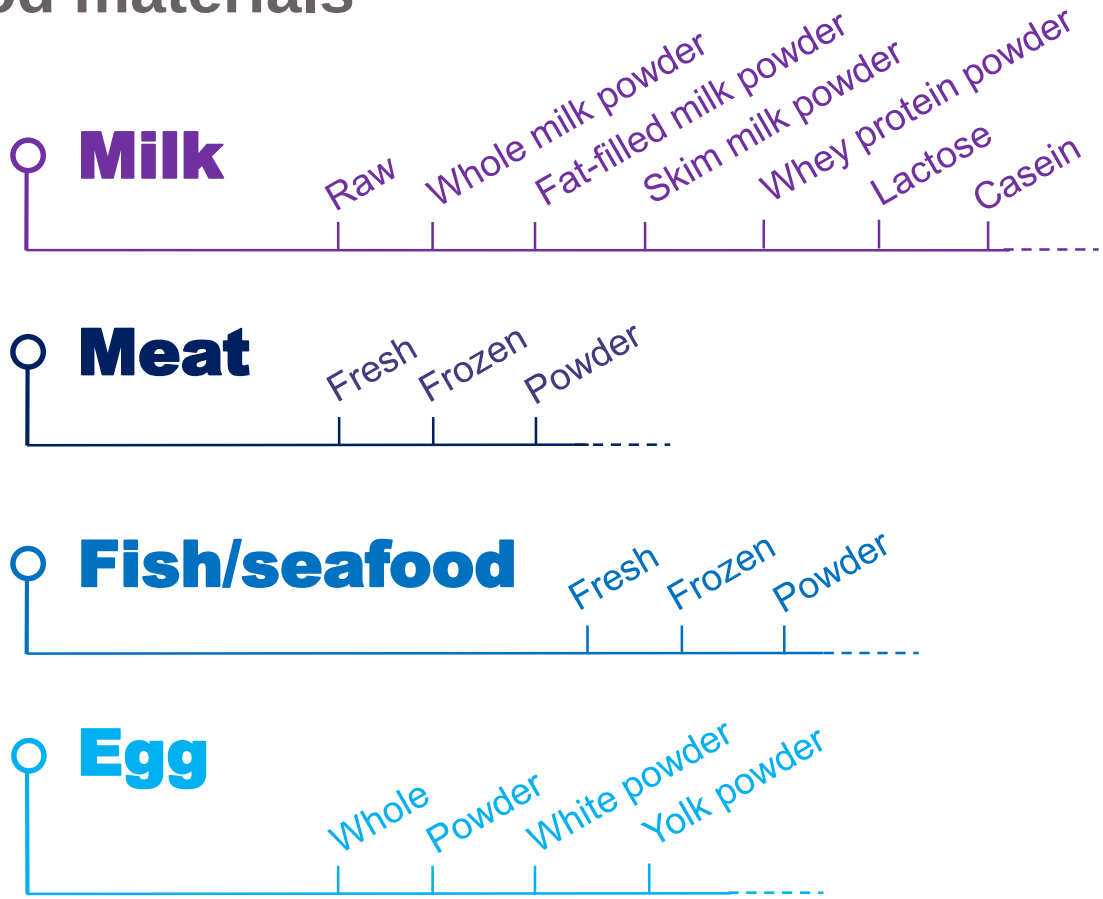
Food materials



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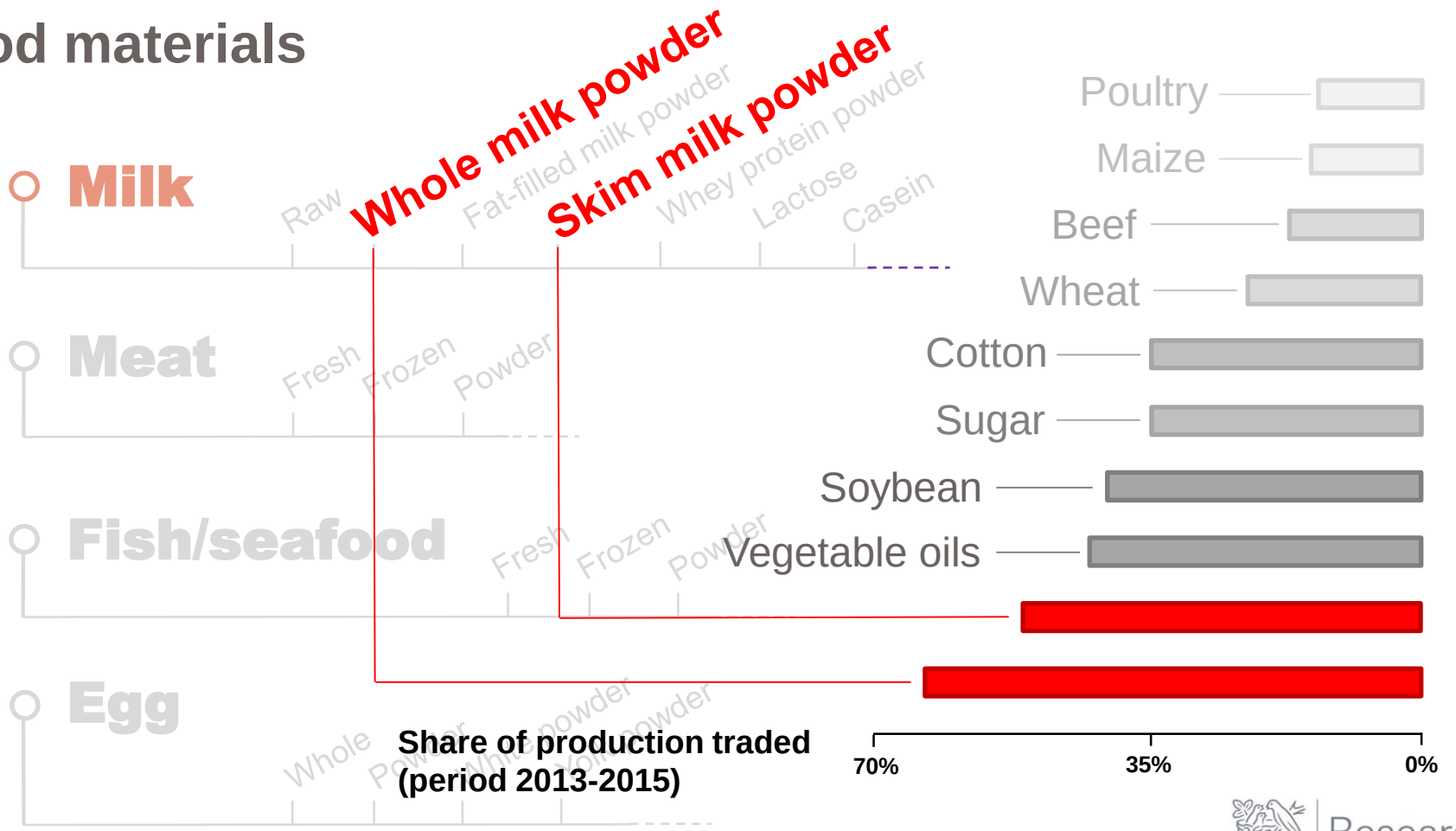
Food materials



Source: OECD/FAO, 2016, OECD-FAO Agricultural Outlook

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Complexity of the supply chain

1 Boulogne-sur-Mer (France)

Findus orders beef-meat lasagnes

2 Metz (France)

Comigel requires ***Tavola*** to manufacture the product

3 Capellen (Luxemburg)

Tavola orders fresh meat to ***Spanghero***

4 Castelnaudary (France)

Spanghero requires a trader to purchase

5 Cyprus

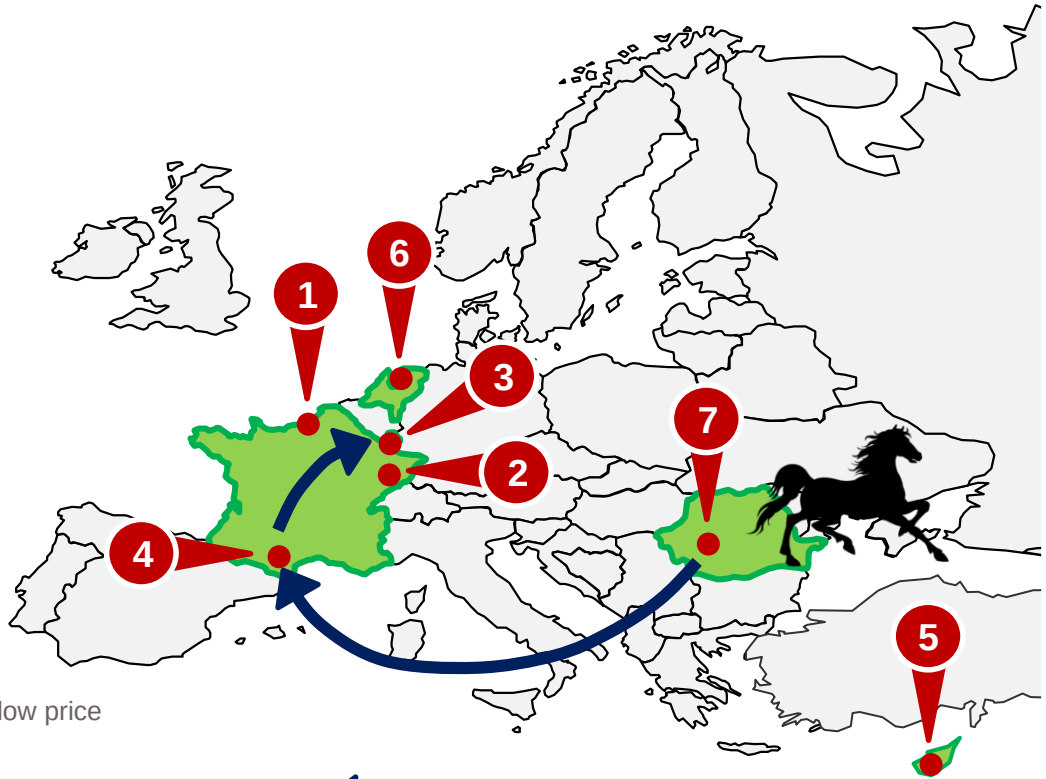
The trader subcontracts the order to another trader in the Netherlands

6 The Netherlands

The Dutch trader orders the fresh meat in Romania at low price

7 Romania

A Romanian slaughterhouse provides ***Spanghero*** with horse meat



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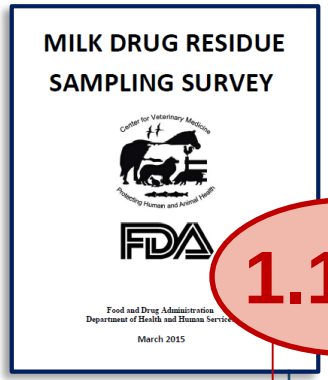
Non-compliant sample rates

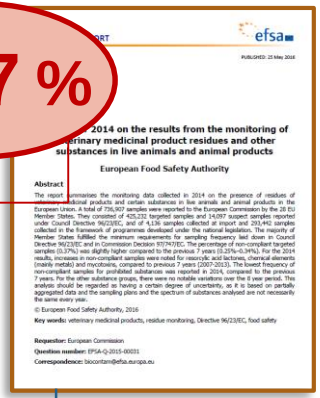


1.15 %

n = 953 samples

0.37 %

n = 736'907 samples



0.81 %

n = 2'600 samples

2015 2016 2018

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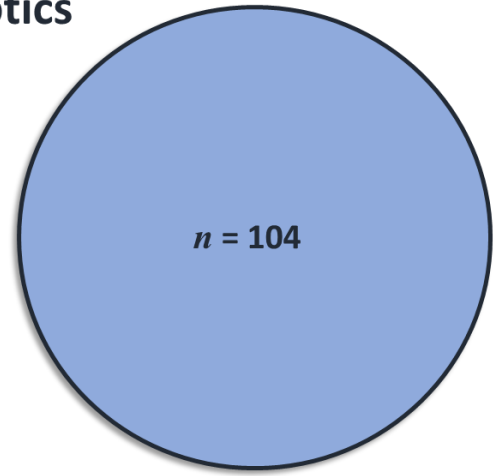
Substances for screening

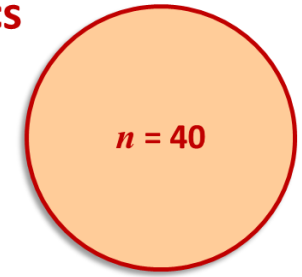
Antibiotics

β-Lactams (23)
Sulfonamides (21)
Quinolones (18)
Aminoglycosides (14)
Tetracyclines (10)
Macrolides (7)
Amphenicols (3)
Diaminopyrimidines (2)
Lincosamides (2)
Rifamycins (2)
Aminocoumarins (1)
Streptogramins (1)



Antiparasitics

Benzimidazoles (14)
Coccidiostats (11)
Avermectins (6)
Salicylanilides (4)
Halogenated phenols (1)
Imidazothiazoles (1)
Organophosphorous compounds (1)
Praziquantel
Pyrantel



Anti-inflammatories

Carprofen
Diclofenac
Flunixin
Meloxicam
Phenylbutazone

Tranquilizers

Carazolol
Chlorpromazine
Xylazine

Source: Delatour *et al.* 2018, *Food Addit. Contam. A*, 35, 632-645.

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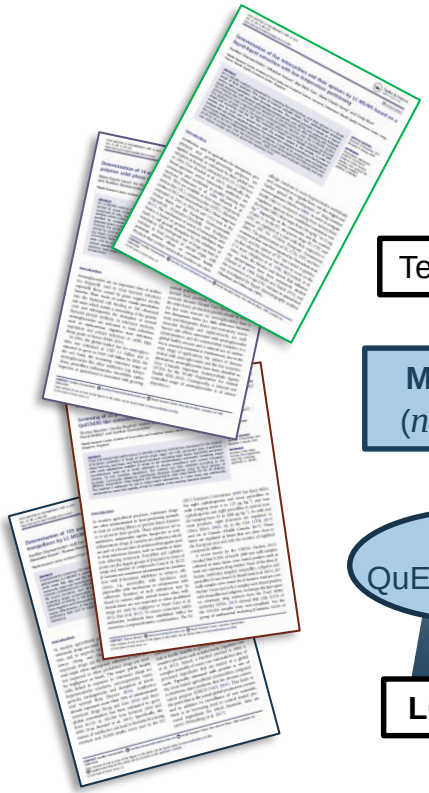
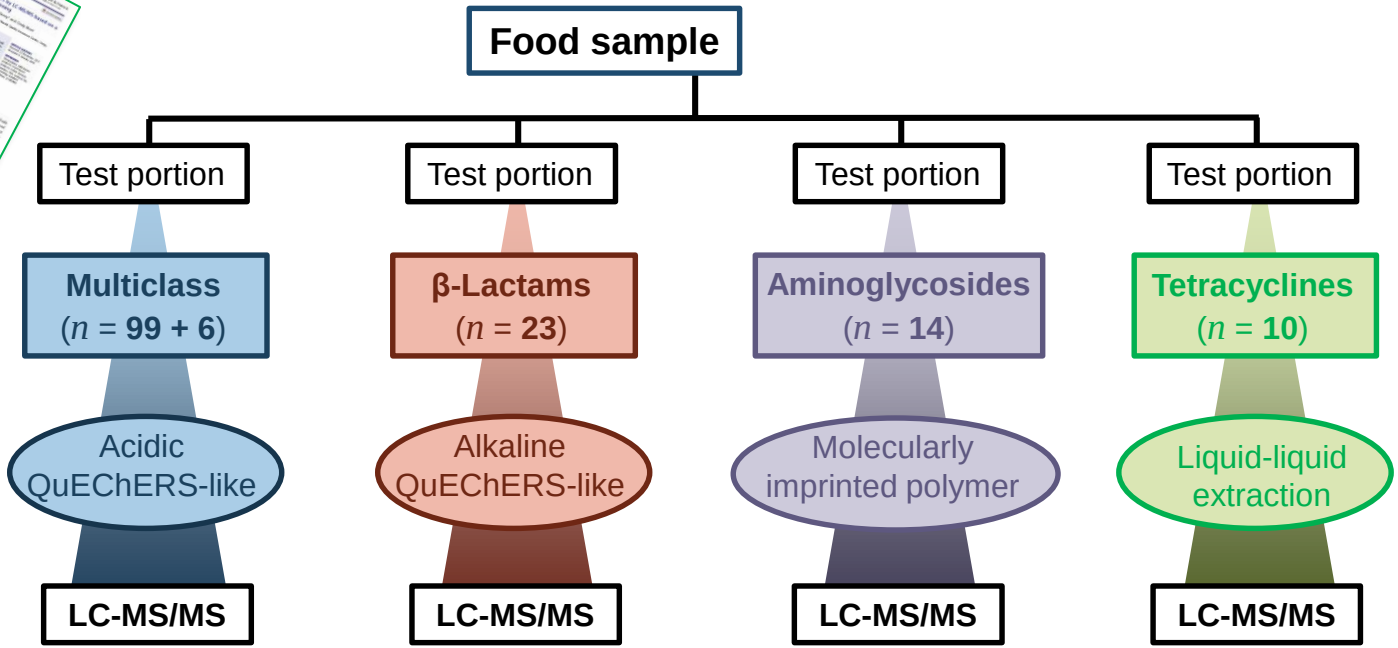
A streamlined LC-MS/MS platform





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A streamlined LC-MS/MS platform

Stability study in standard solutions

FOOD ADDITIVES & CONTAMINANTS: PART A, 2018
VOL. 35, NO. 4, 632–646
https://doi.org/10.1080/19440049.2018.1433383

Taylor & Francis
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Check for updates

Stability study of veterinary drugs in standard solutions for LC-MS/MS screening in food

Aurélien Desmarchelier*, Philipp Feuerstein*, Hassan Fathi Ahmed*, Julie Moulin*, Andrea Beck*, Claudia Mujahid*, Thomas Bessaire*, Marie-Claude Savoy* and Pascal Mottier*

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& Background Challenges

FOOD ADDITIVES & CONTAMINANTS: PART A, 2018
VOL. 35, NO. 4, 632–646
https://doi.org/10.1080/19440049.2018.1426890

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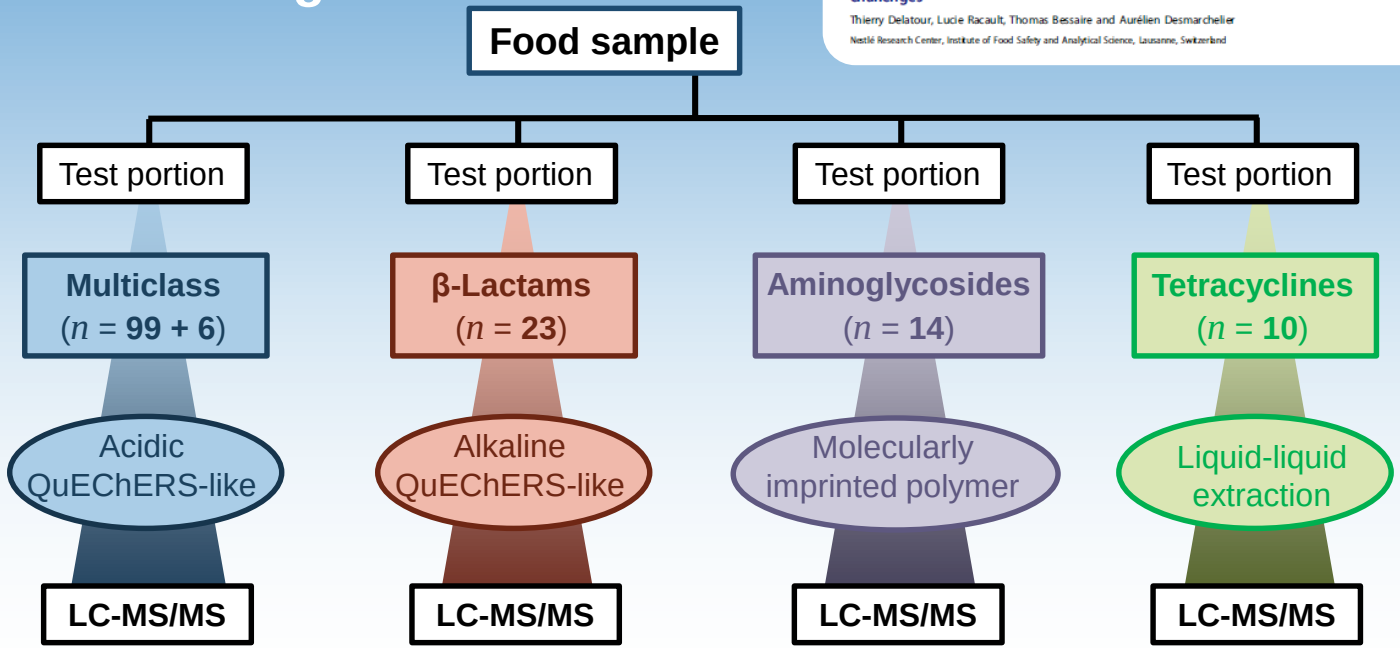
Check for updates

Screening of veterinary drug residues in food by LC-MS/MS. Background and challenges

Thierry Delatour, Lucie Racault, Thomas Bessaire and Aurélien Desmarchelier

Nestlé Research Center, Institute of Food Safety and Analytical Science, Lausanne, Switzerland



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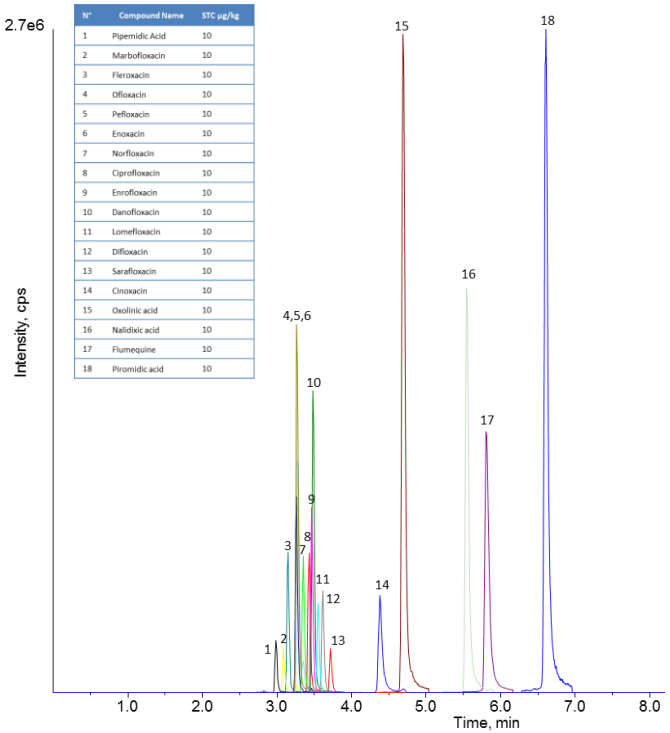
Stream ‘Multiclass’

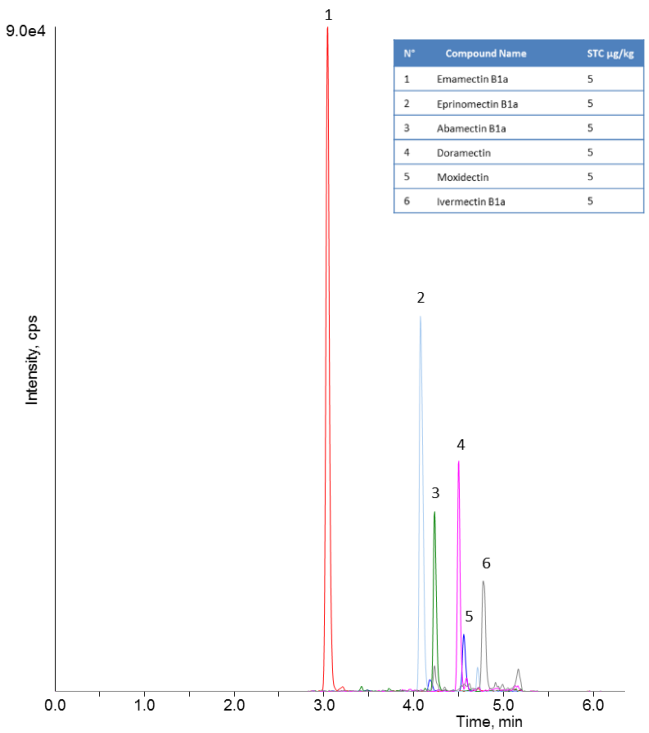
1xSTC (screening target concentration)

Quinolones in beef

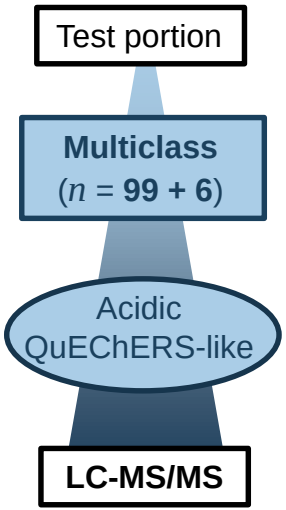


N°	Compound Name	STC µg/kg
1	Pipemidic Acid	10
2	Marbofloxacin	10
3	Fleroxacin	10
4	Ofloxacin	10
5	Pefloxacin	10
6	Enoxacin	10
7	Norfloxacin	10
8	Ciprofloxacin	10
9	Enrofloxacin	10
10	Danofloxacin	10
11	Lomefloxacin	10
12	Difloxacin	10
13	Sarafloxacin	10
14	Cinoxacin	10
15	Oxolinic acid	10
16	Nalidixic acid	10
17	Flumequine	10
18	Piromidic acid	10

Avermectins in salmon



N°	Compound Name	STC µg/kg
1	Emamectin B1a	5
2	Eprinomectin B1a	5
3	Abamectin B1a	5
4	Doramectin	5
5	Moxidectin	5
6	Ivermectin B1a	5



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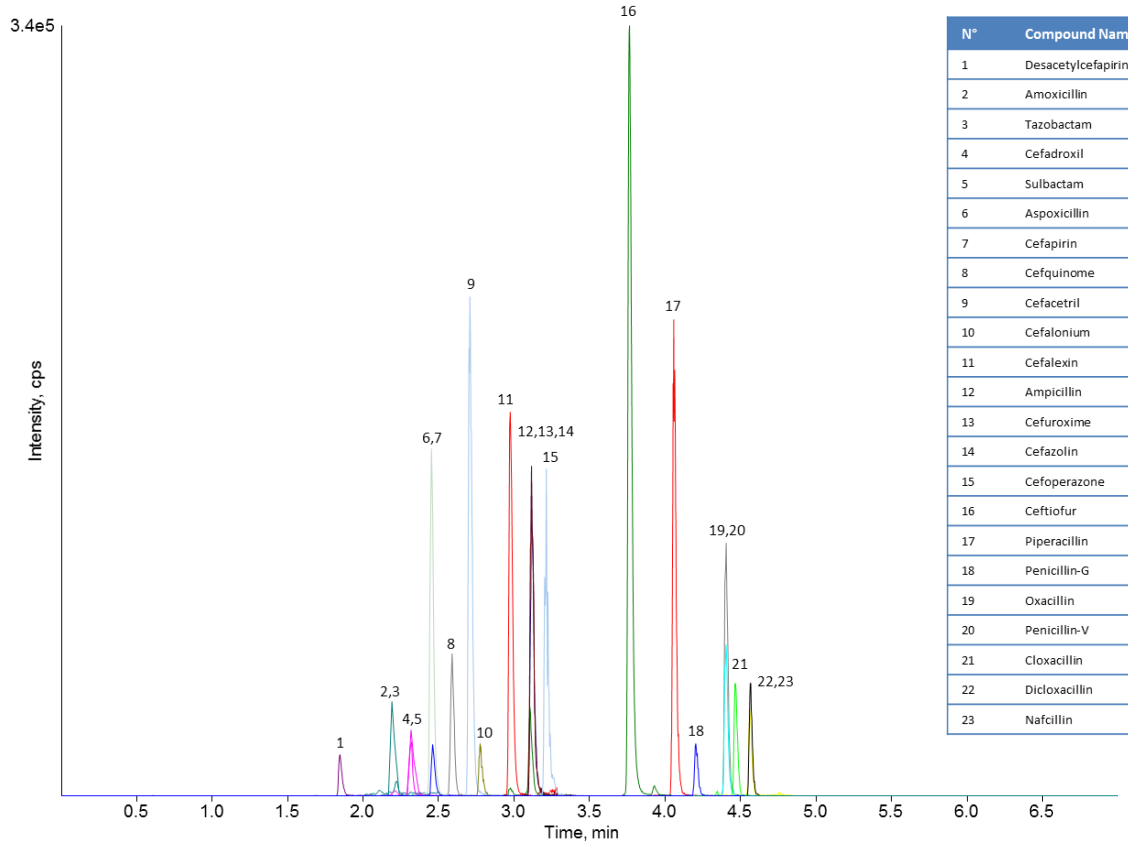
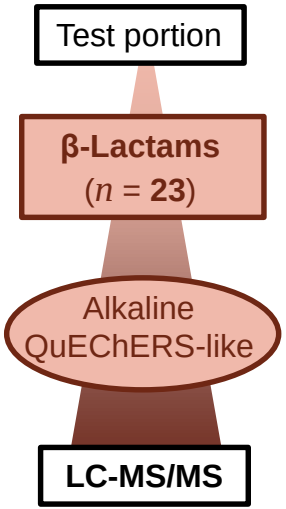
Stream ‘β-Lactams’

Skimmed milk powder at 1xSTC



N°	Compound Name	STC µg/kg
1	Desacetylcefapirin	20
2	Amoxicillin	4
3	Tazobactam	25
4	Cefadroxil	50
5	Sulbactam	25
6	Aspoxicillin	25
7	Cefapirin	10
8	Cefquinome	10
9	Cefacetril	50
10	Cefalonium	10
11	Cefalexin	50
12	Ampicillin	4
13	Cefuroxime	50
14	Cefazolin	50
15	Cefoperazone	50
16	Ceftiofur	50
17	Piperacillin	10
18	Penicillin-G	4
19	Oxacillin	10
20	Penicillin-V	10
21	Cloxacillin	10
22	Dicloxacillin	10
23	Nafcillin	5



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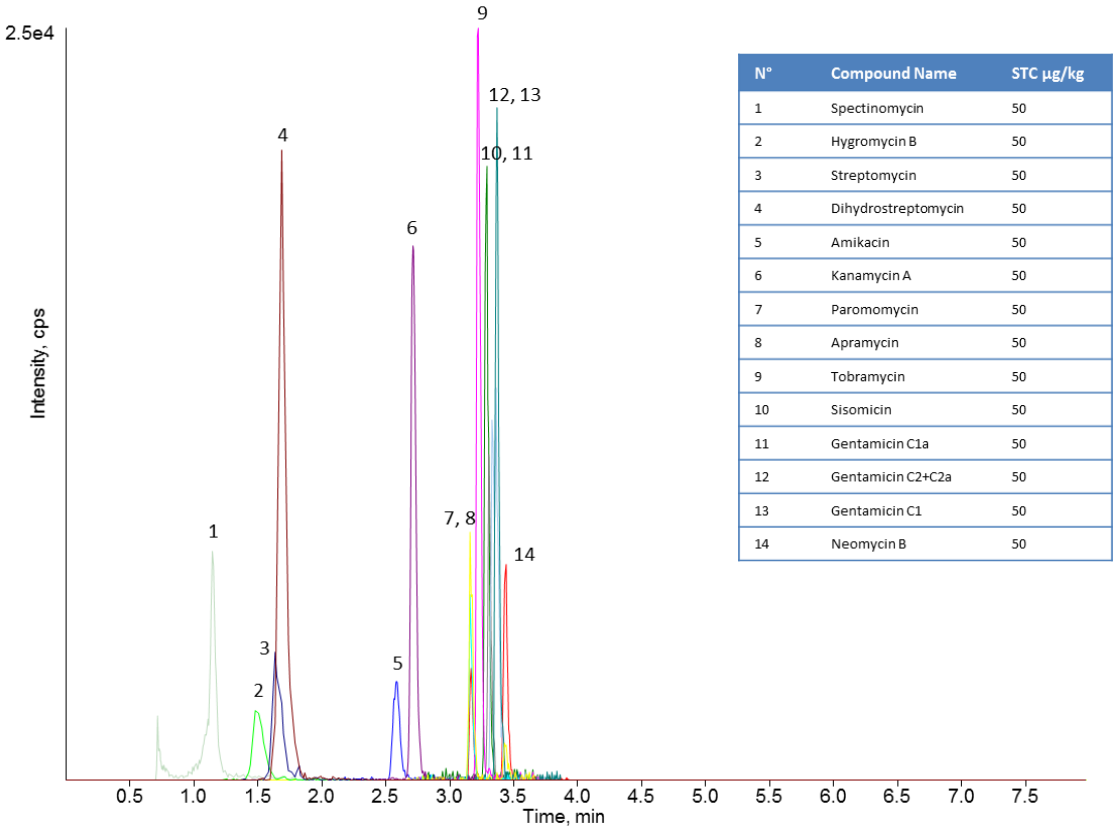
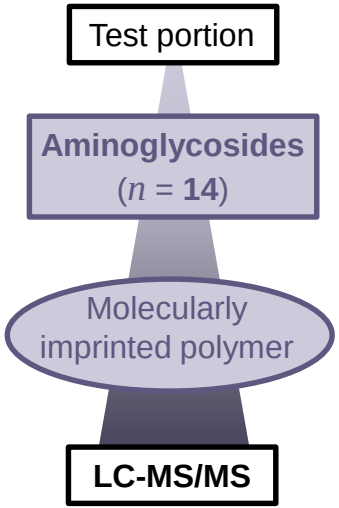
Stream ‘Aminoglycosides’

Egg powder at 1xSTC



N°	Compound Name	STC µg/kg
1	Spectinomycin	50
2	Hygromycin B	50
3	Streptomycin	50
4	Dihydrostreptomycin	50
5	Amikacin	50
6	Kanamycin A	50
7	Paromomycin	50
8	Apramycin	50
9	Tobramycin	50
10	Sisomicin	50
11	Gentamicin C1a	50
12	Gentamicin C2+C2a	50
13	Gentamicin C1	50
14	Neomycin B	50



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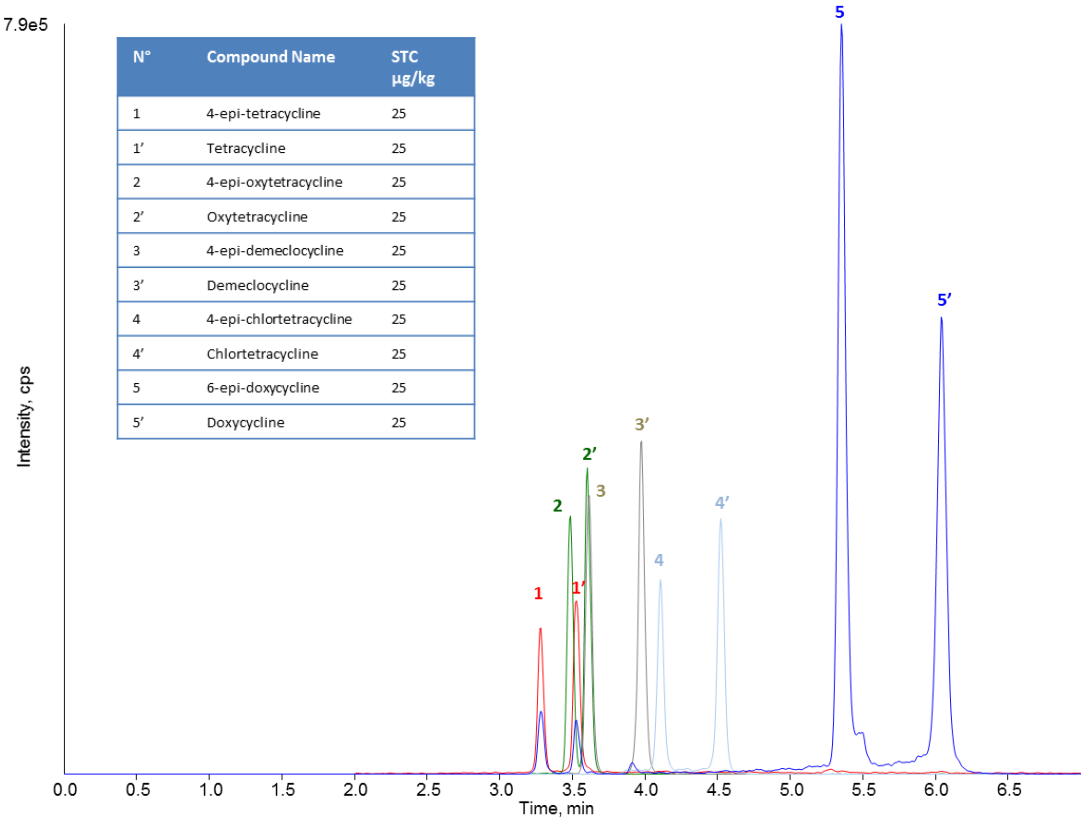
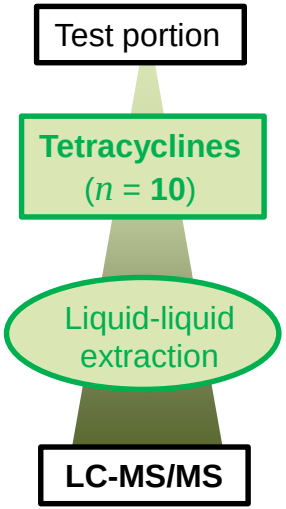
Stream ‘Tetracyclines’

Meat-based babyfood at 1xSTC

N°	Compound Name	STC µg/kg
1	4-epi-tetracycline	25
1’	Tetracycline	25
2	4-epi-oxytetracycline	25
2’	Oxytetracycline	25
3	4-epi-demeclocycline	25
3’	Demeclocycline	25
4	4-epi-chlortetracycline	25
4’	Chlortetracycline	25
5	6-epi-doxycycline	25
5’	Doxycycline	25





What’s screening about?

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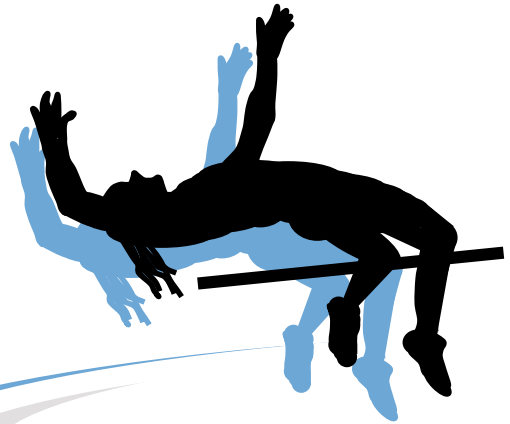
What's screening about?



Absent *versus* **Present**

Low *versus* **High**

Compliant *versus* **Violative**

Free *versus* **Suspect**

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Variation is a challenge for electrospray!

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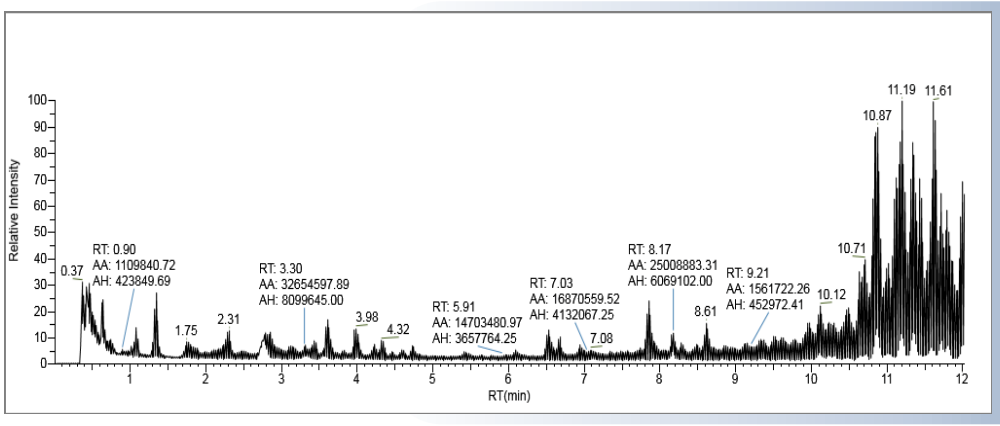
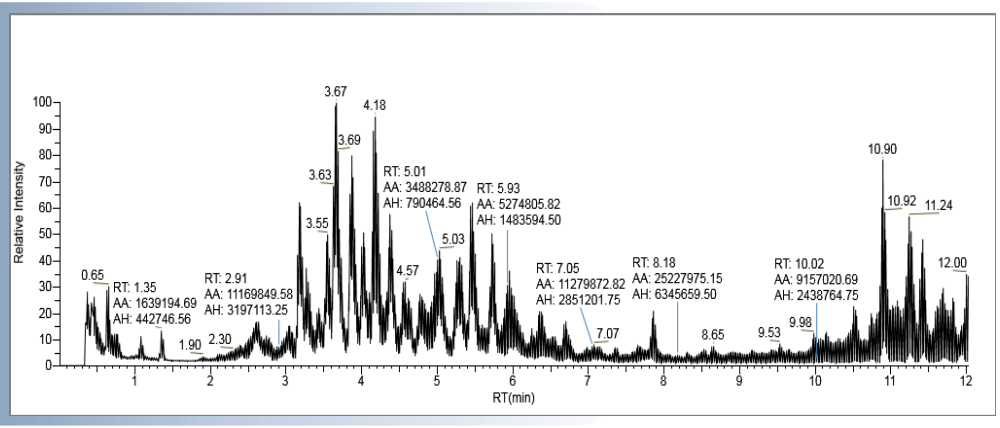
Variation of food composition

Pork powder



Hypoallergenic infant formula



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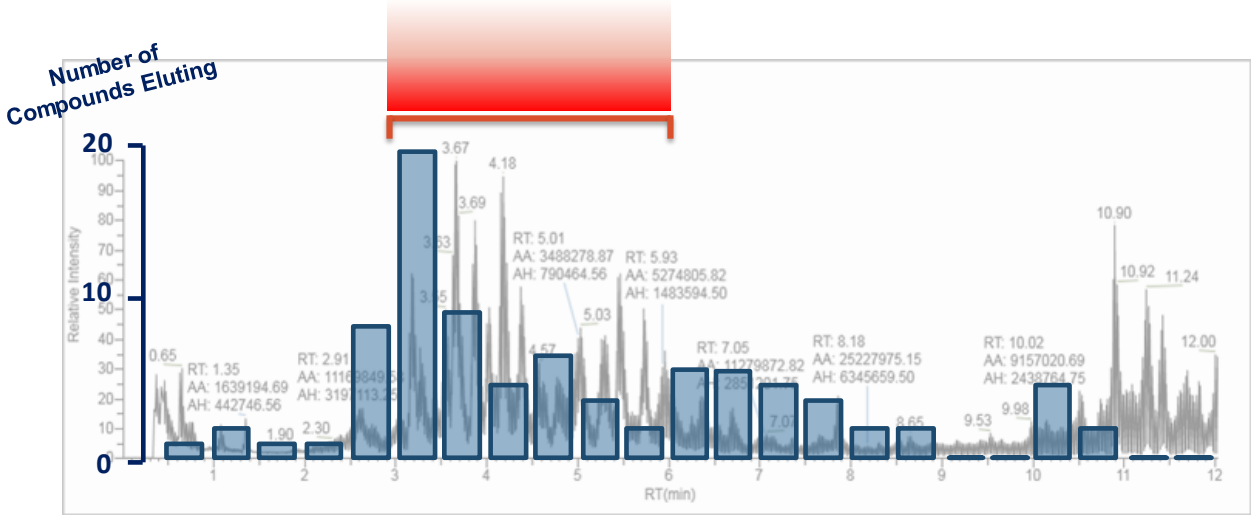
Interference with the matrix

56% of the analytes
elutes in the range 3-6 min



Hypoallergenic infant formula

Process efficiency (PE)

$$\text{Rec}_{Abs}\,(\%) = \text{Absolute recovery} = \frac{\text{Area}_{Sample}}{\text{Area}_{Extract}}$$

$$\text{ME}\,(\%) = \text{Matrix effect} = \frac{\text{Area}_{Extract}}{\text{Area}_{Standard}}$$

$$\text{PE}\,(\%)$$

Area_{Sample}
Signal of a sample spiked before extraction

$\text{Area}_{Extract}$
Signal of a sample spiked in the extract

$\text{Area}_{Standard}$
Signal of the chemical standard

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Process efficiency (PE)

$$\text{Rec}_{Abs}\,(\%) \times \text{ME}\,(\%) = \text{PE}\,(\%)$$

Absolute recovery $= \dfrac{\text{Area}_{Sample}}{\text{Area}_{Extract}}$

Matrix effect $= \dfrac{\text{Area}_{Extract}}{\text{Area}_{Standard}}$

Area_{Sample}
Signal of a sample spiked before extraction

$\text{Area}_{Extract}$
Signal of a sample spiked in the extract

$\text{Area}_{Standard}$
Signal of the chemical standard

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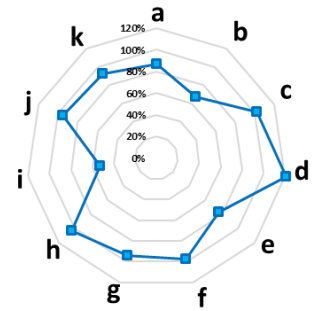
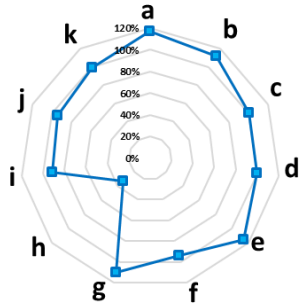
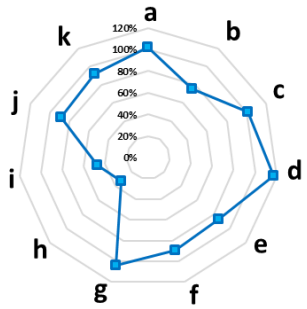
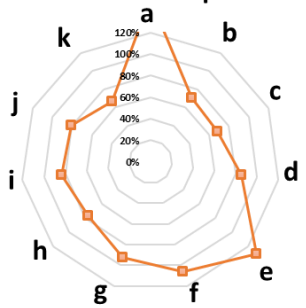
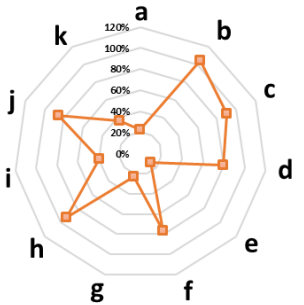
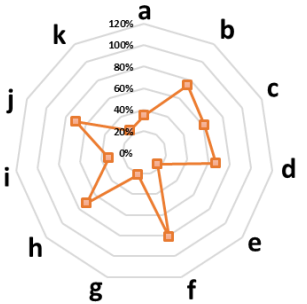
Process efficiency (PE)

	Rec_{Abs} (%)	ME (%)	PE (%)
Rifaximin			
Amprolium			

Infant formulae: **a**, **b**, **c**

Milk powders: **d**, **e**

Baby food: **f**

Meat powder: **g**

Fresh meat: **h**, **i**

Fish: **j**

Infant cereal: **k**

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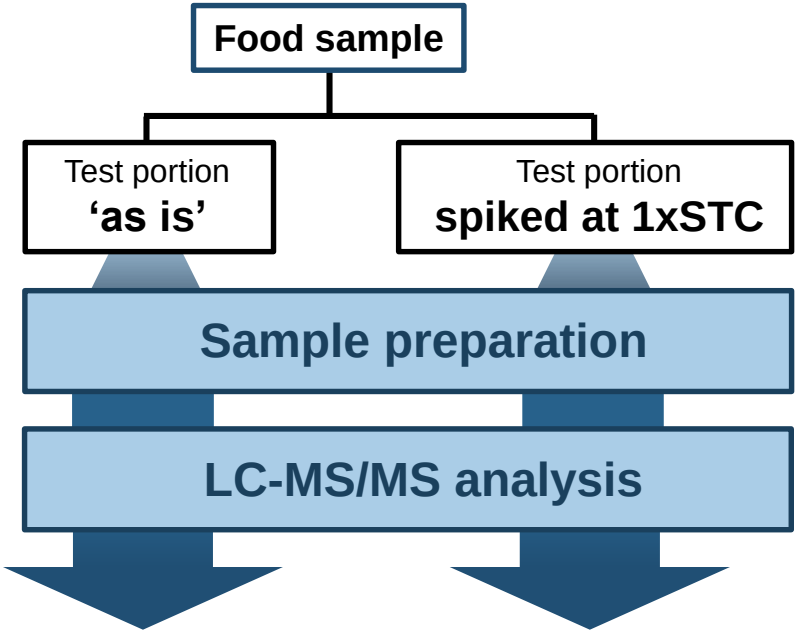
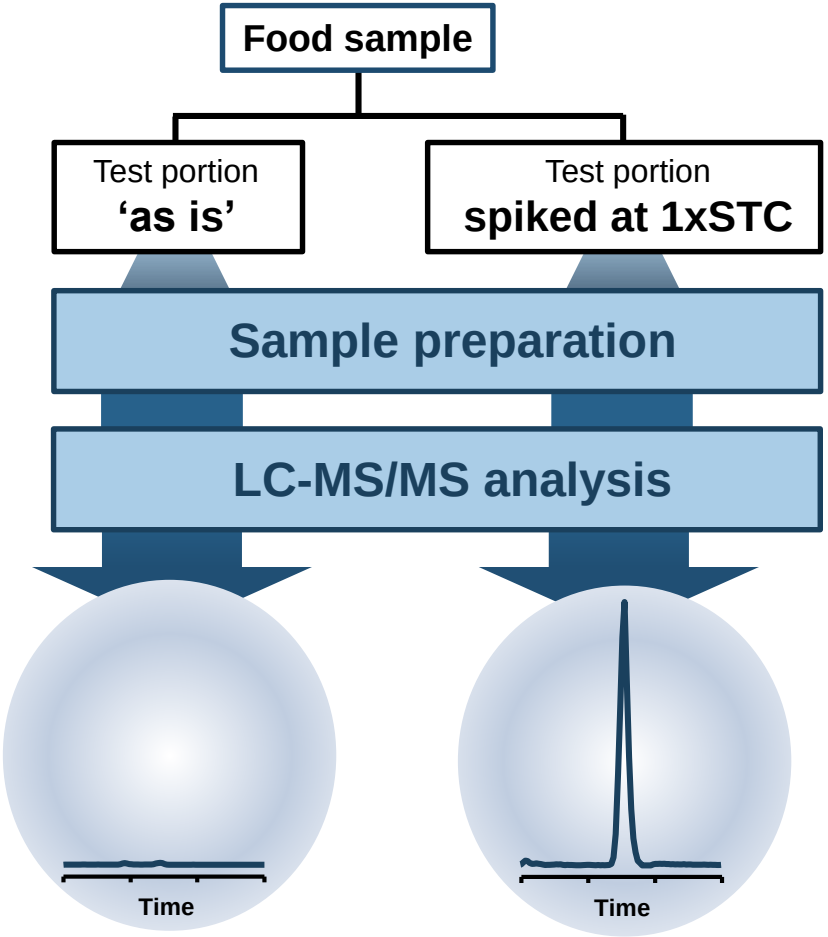
Sample workflow

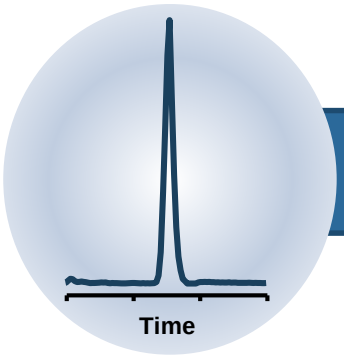


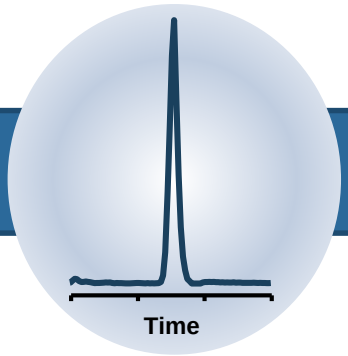
Sample workflow

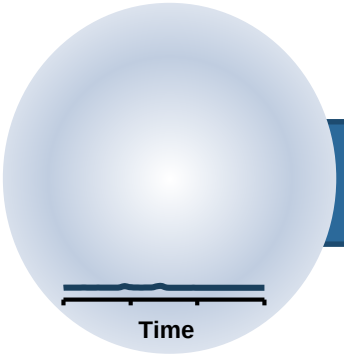


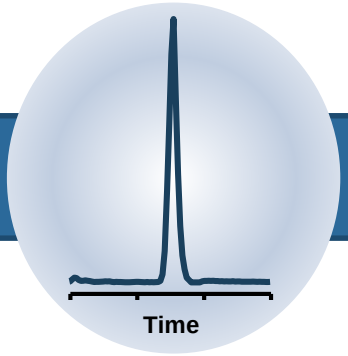
Effective & reliable review of the data

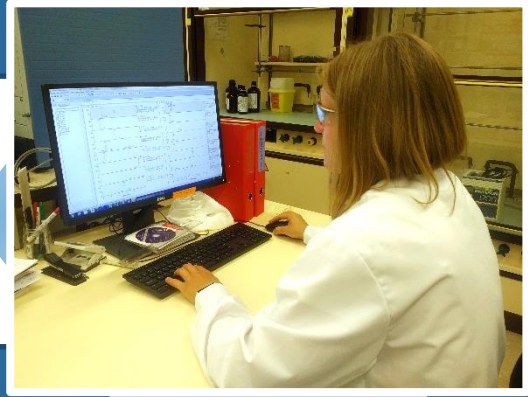
‘As is’ **Spiked at 1xSTC**











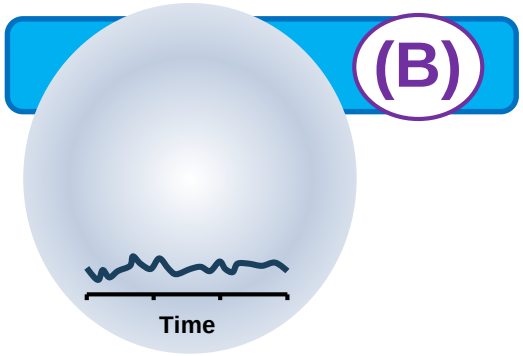
Rejected

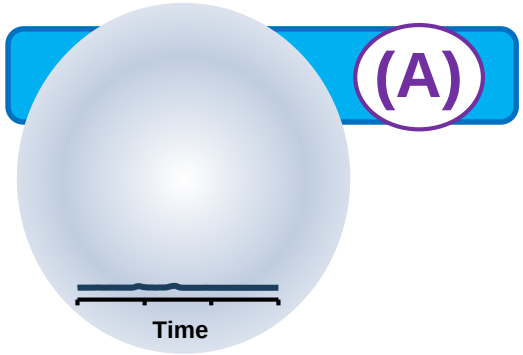
Approved

Nestlé Research

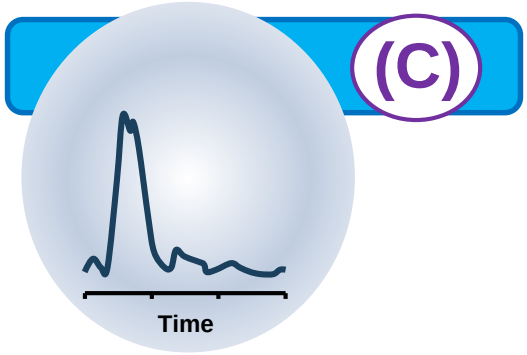
Effective & reliable review of the data

‘As is’





‘As is’

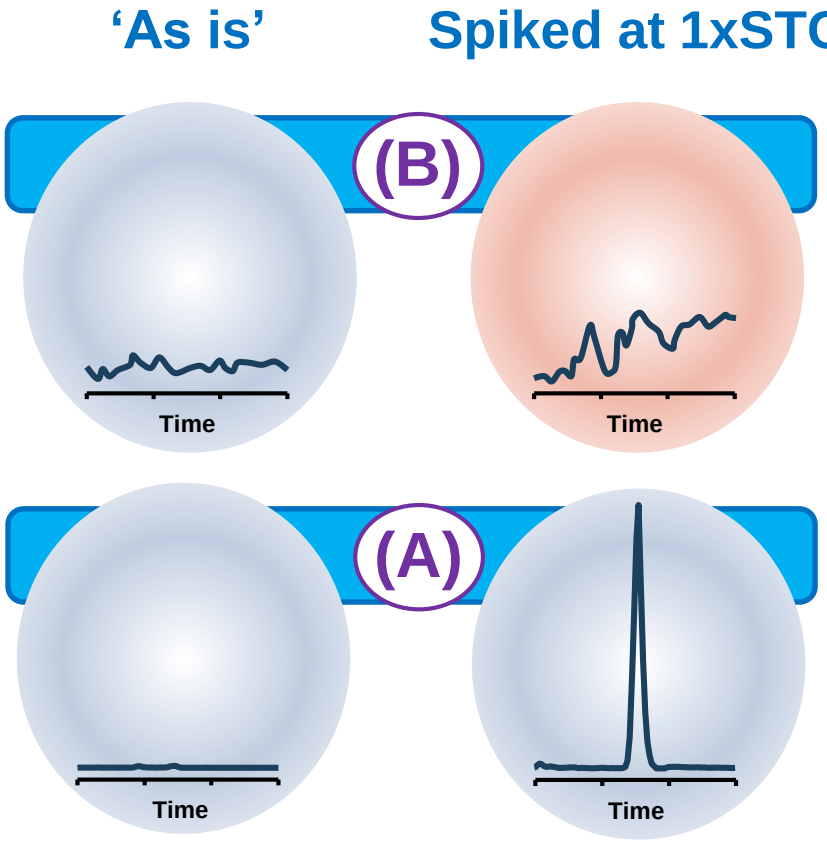


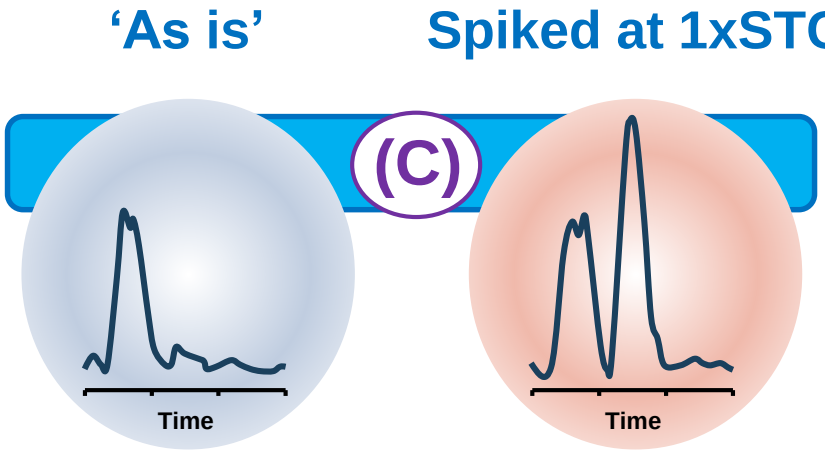
(A): Sample is negative

(B): Sample is negative

(C): Sample is positive

Effective & reliable review of the data



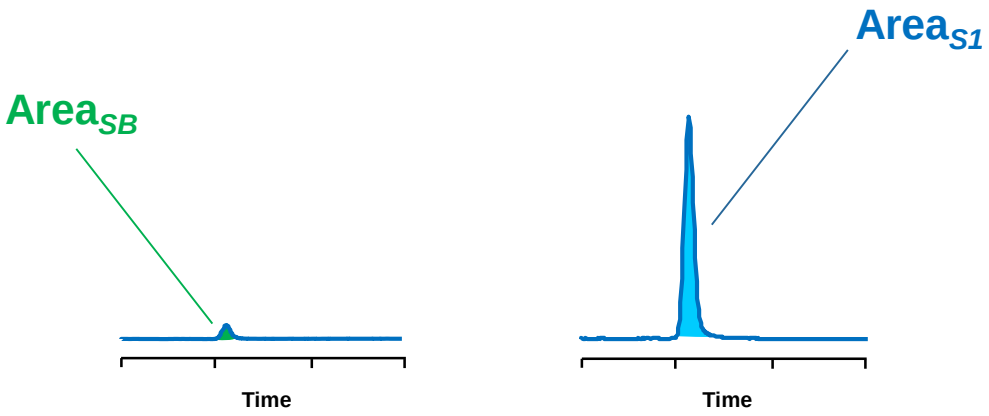


(A): Sample is negative

(B): Sample has an odd behavior

(C): Sample is negative

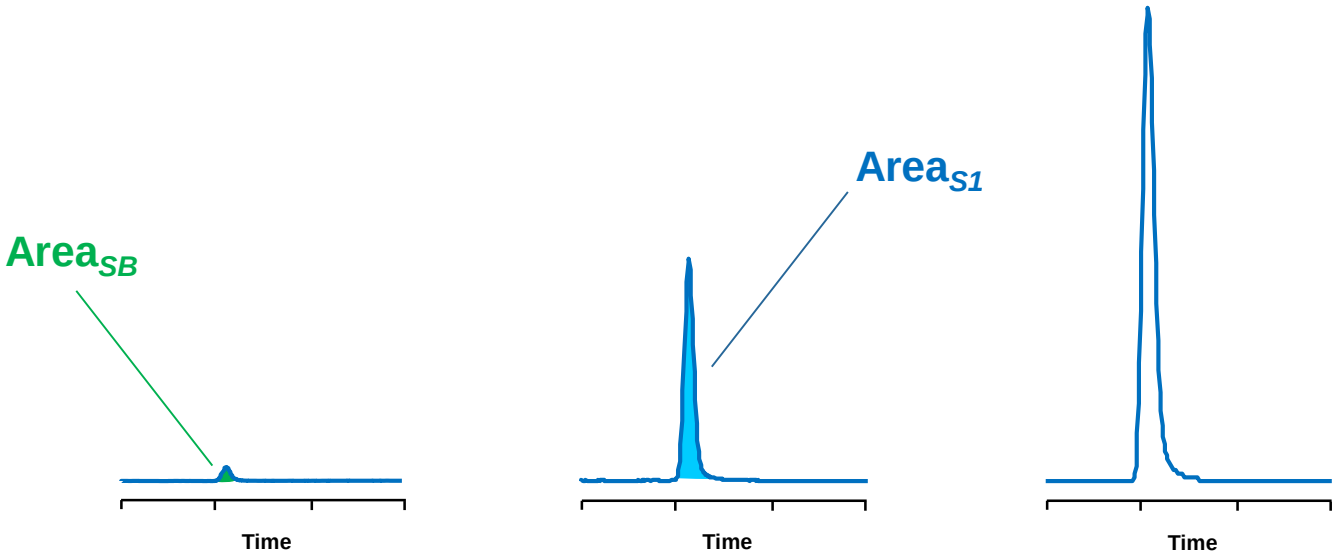
Screening based on absolute areas



Sample ‘as is’
(unspiked)

Spiked at 1XSTC

Screening based on area ratios

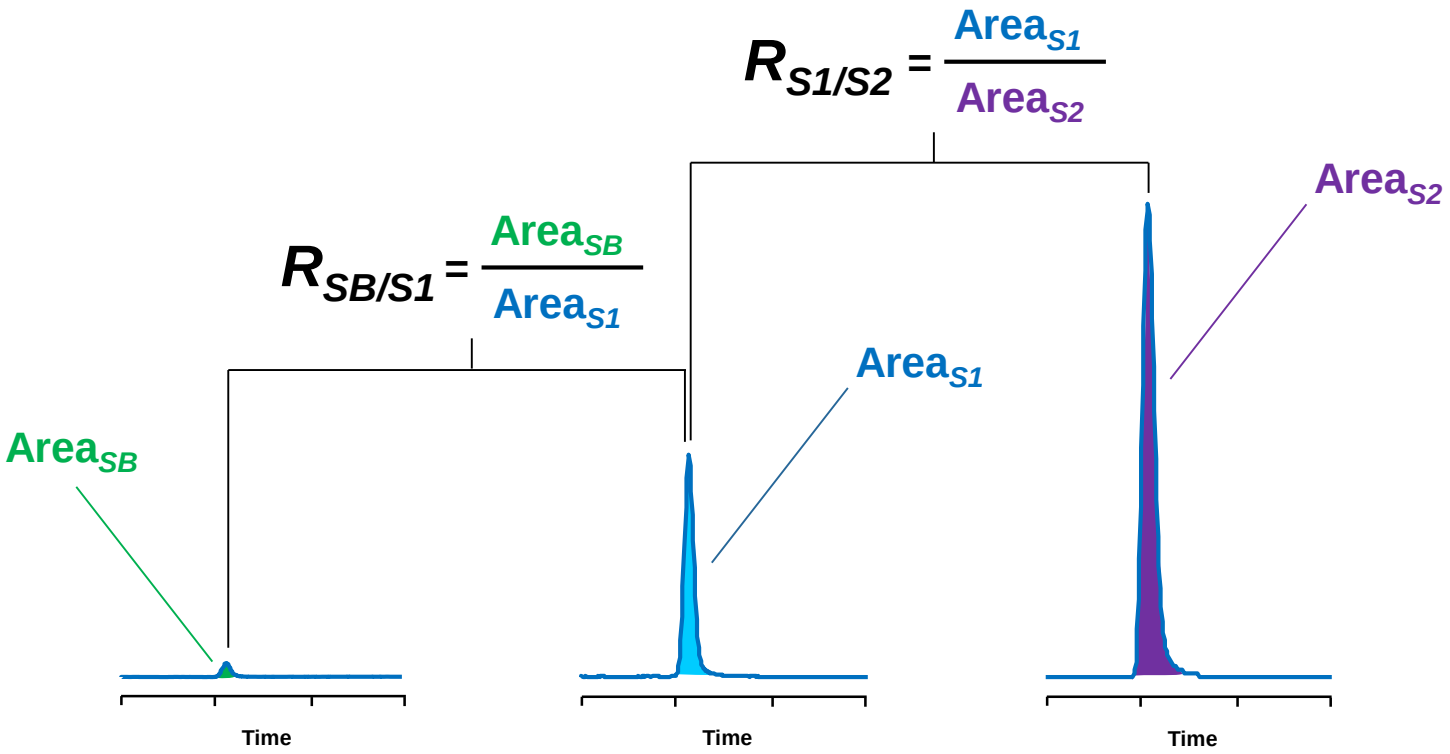


Sample ‘as is’
(unspiked)

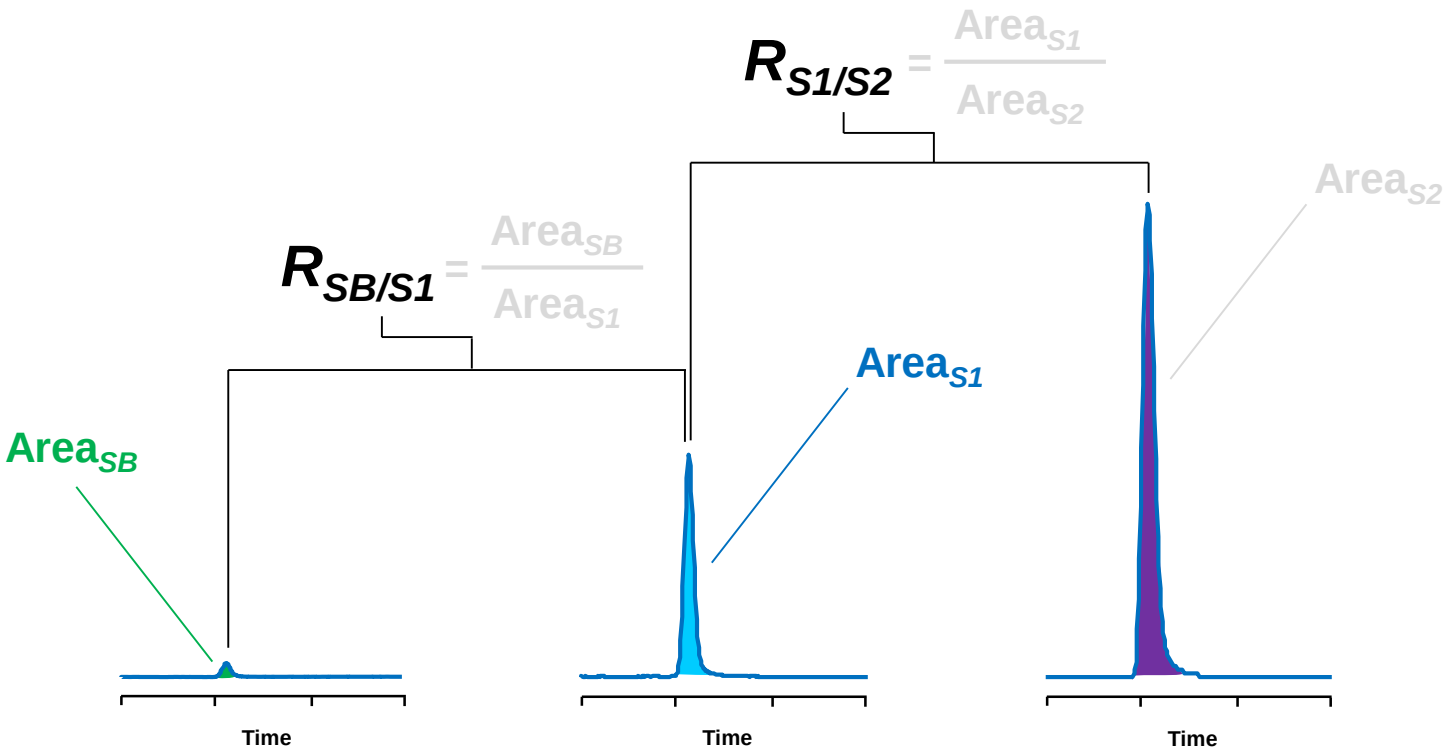
Spiked at 1XSTC

Spiked at 2XSTC

Screening based on area ratios



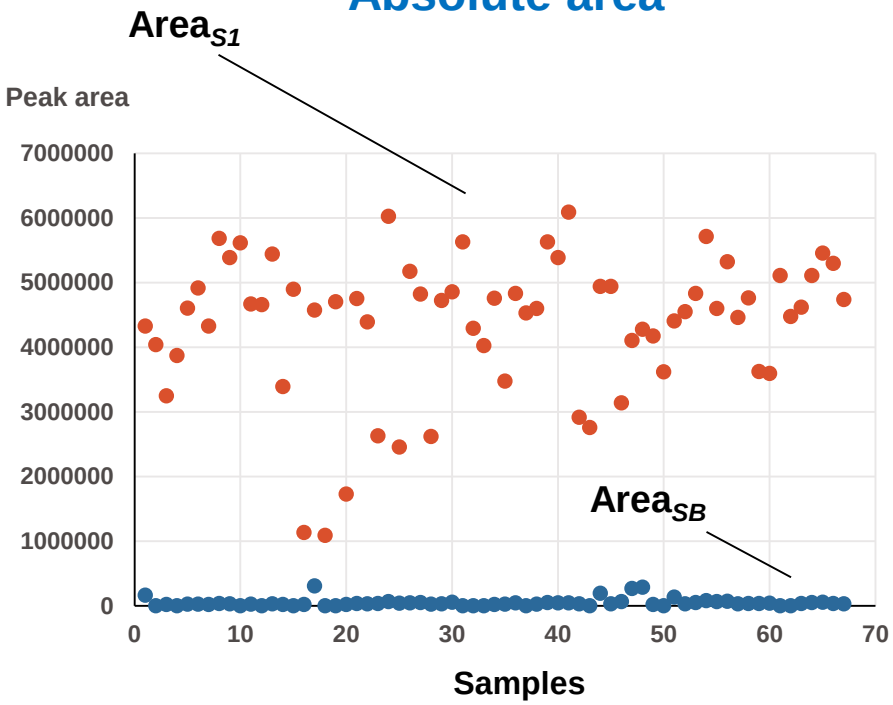
Assessment of screening performance



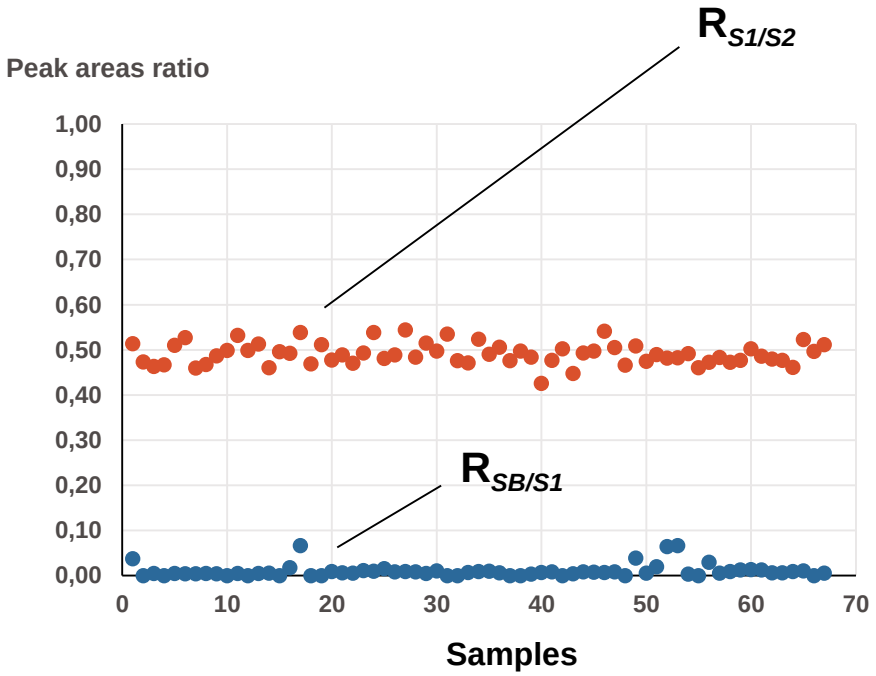
Signal response in food samples

5-Hydroxythiabendazole

Absolute area



Area ratio



Sample at 1xSTC

Blank sample

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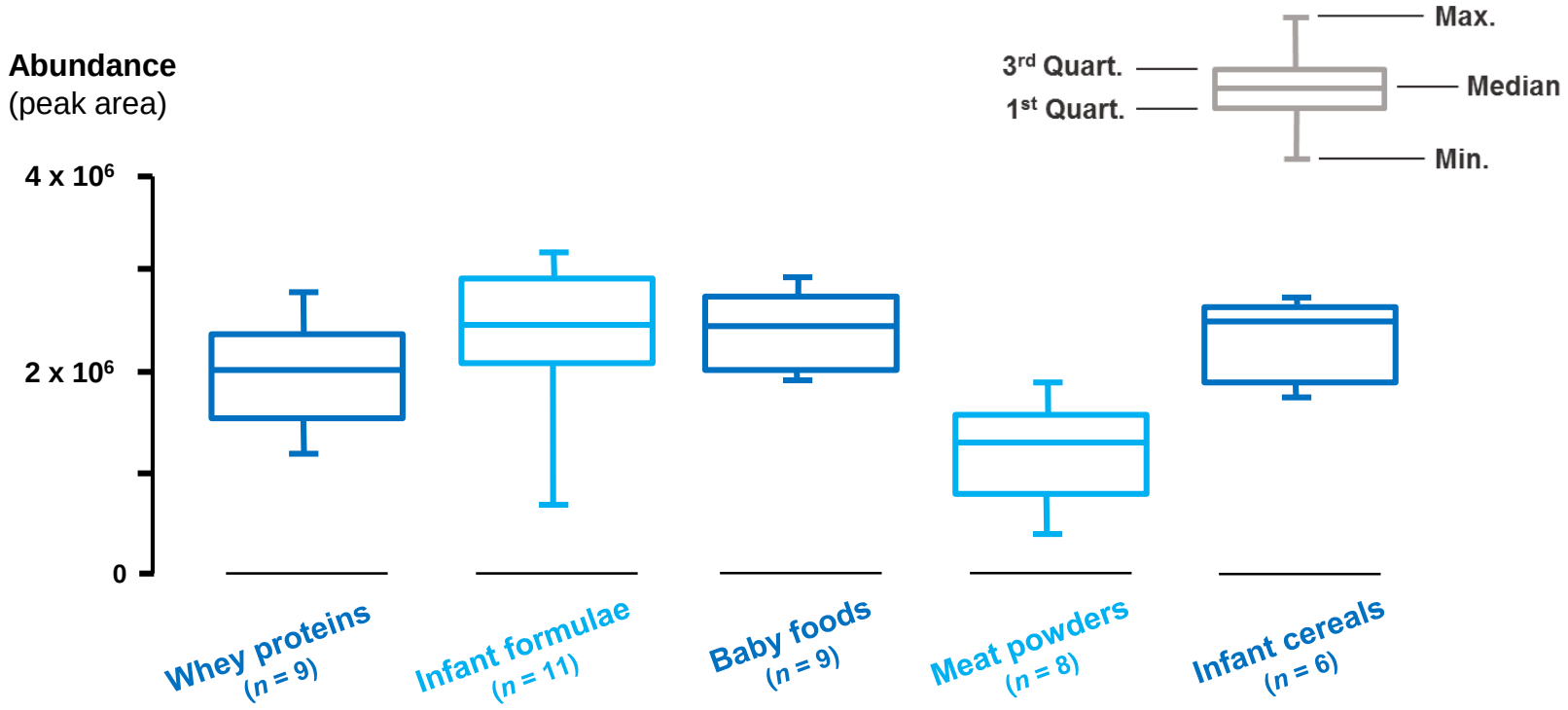
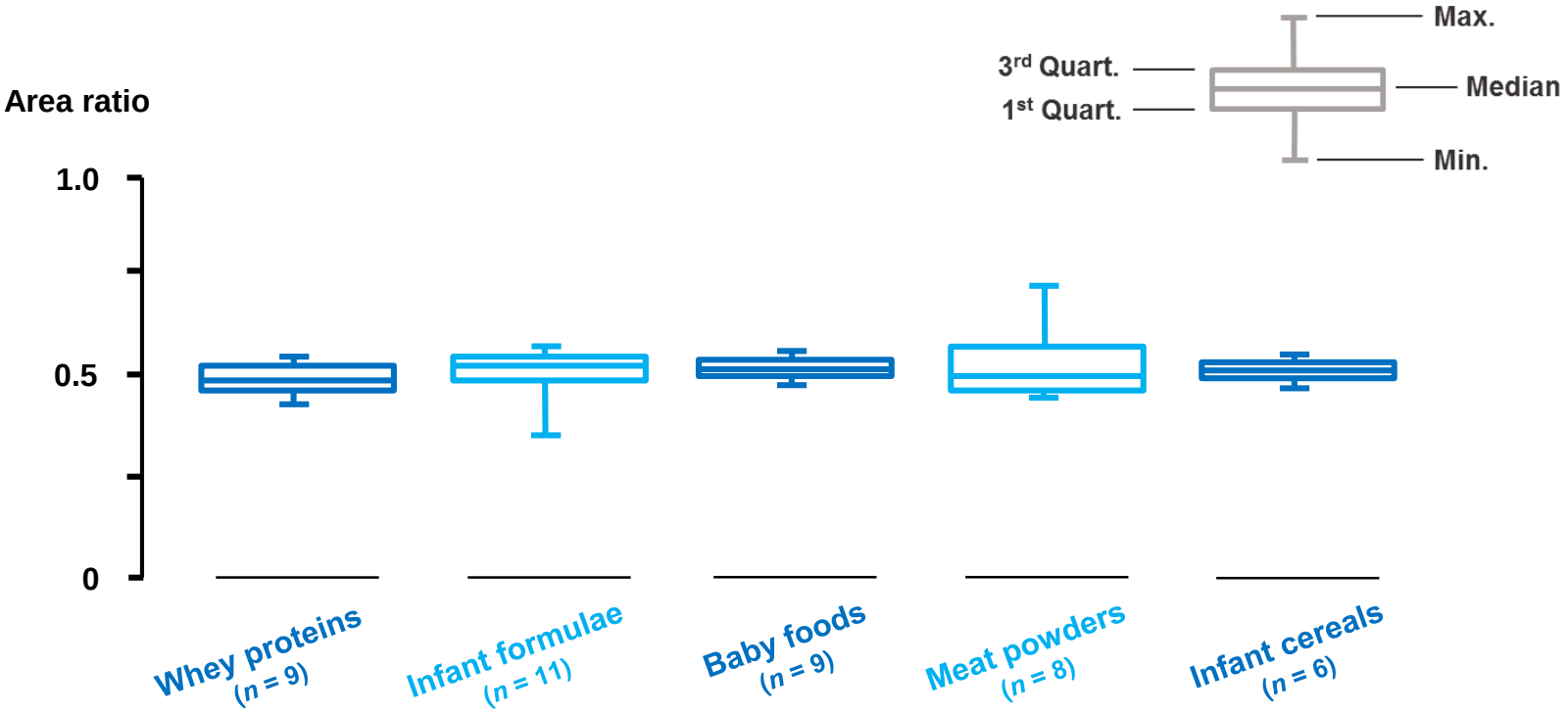
Response per category – Screening with absolute area



5-Hydroxythiabendazole at 1xSTC

Nestlé Research

Response per category – Screening with area ratio



5-Hydroxythiabendazole at 1xSTC

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From variation within a category …

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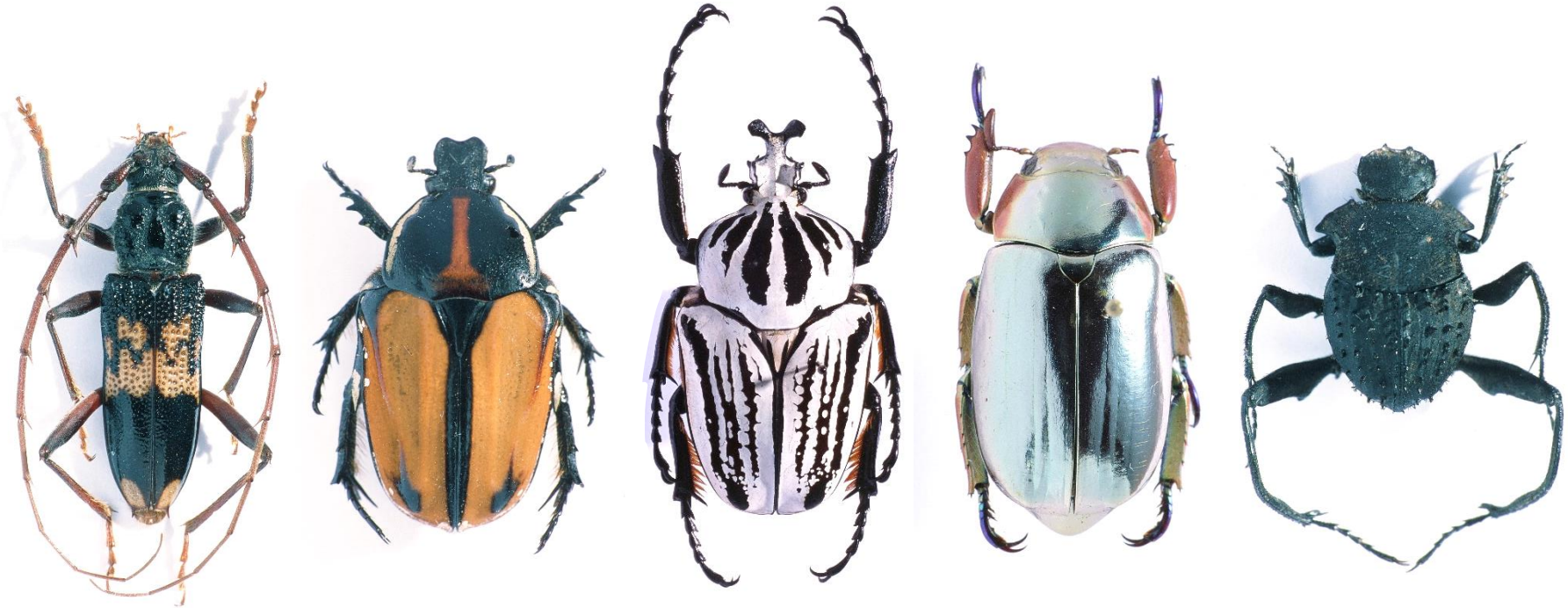
… to variation across categories



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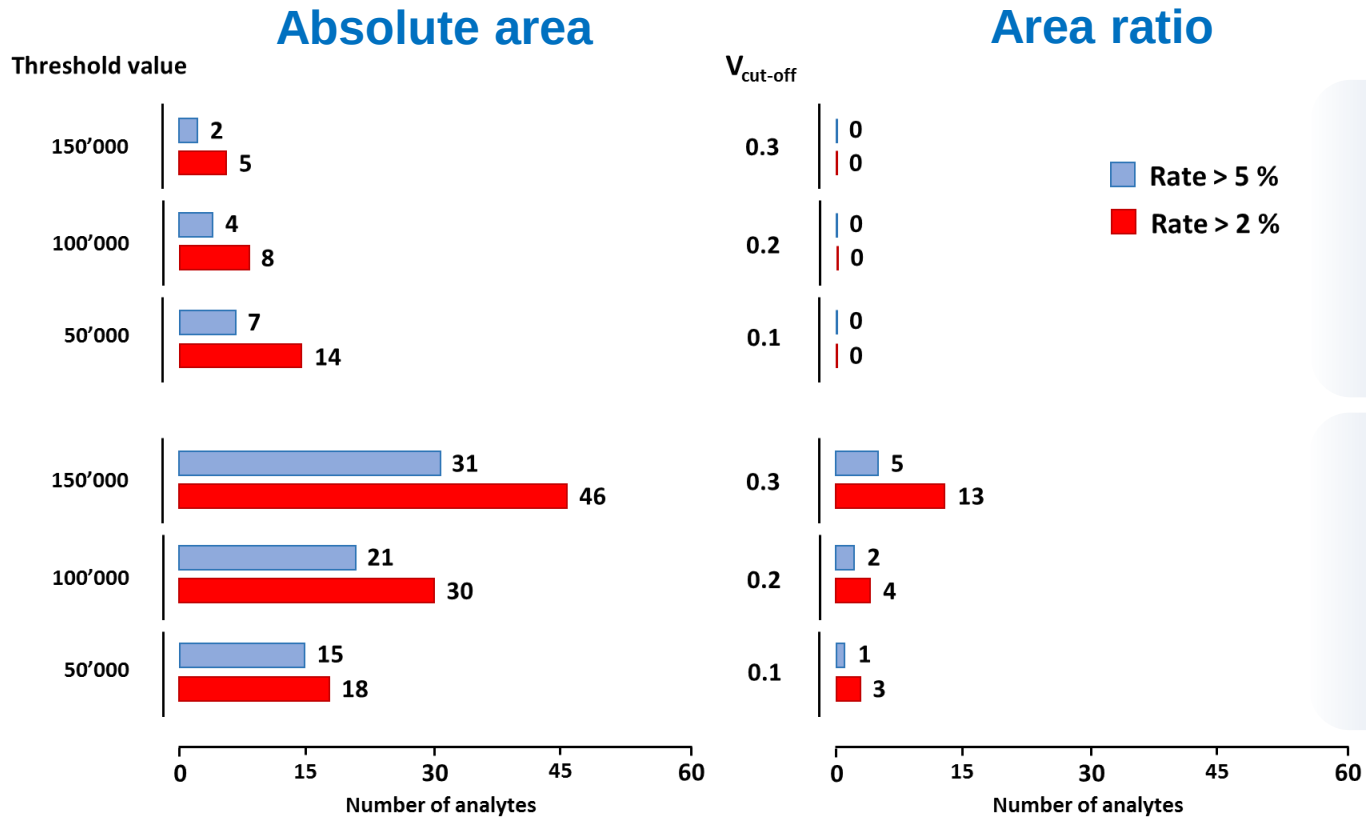
False response rates (*n* = 105 analytes)



False positives

False negatives

Source: Delatour *et al.* 2018, *Food Control*, 94, 353-360.

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Take home message

- Liquid chromatography-mass spectrometry is a suitable technique for multiresidue screening of more than a hundred veterinary drug residues in food.
- Instability of electrospray mediated by food composition variation is a limitation for screening in a broad range of commodities.

- The ‘Area ratio’ approach improves the efficiency and the reliability of the data review.
- The ‘Area ratio’ approach significantly improves the screening performance in terms of false responses.

Thanks for your attention



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